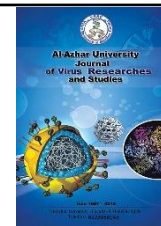




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Impact of Calcium Channel Blockers on Murine Trichinellosis

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Abstract

Essentially, *Trichinella spiralis* is considered to be a re-emerging intestinal and tissue parasitic nematode that causes serious foodborne infections worldwide. To evaluate the role of a commonly used Calcium Channel Blocker (CCB) (nifedipine) on muscular phase of murine trichinellosis utilizing parasitological and histopathological parameters. The study was carried out on 45 Swiss albino mice. The infection was done orally for the infected groups with about 250 ± 50 *Trichinella spiralis* larvae (TSL). Sacrification of mice was done on the 35th day post-infection (PI). Detection and counting the number of larvae in the diaphragm of mice of each group were done microscopically and specimens were taken and fixed in 10% formalin to detect histopathological changes. The study revealed that nifedipine has reduction rate (67.96%) on *Trichinella* larvae count in diaphragm. Histopathological examination revealed mild reduction of inflammatory reactions. Nifedipine has been concluded to be used in the treatment of trichinellosis, to reduce the number of larvae and to improve pathological changes.

Keywords: *Trichinella spiralis*, CCB (nifedipine), Muscular phase.

1. Introduction

Trichinellosis, a parasitic disease caused by *Trichinella spp.*, most often *Trichinella spiralis* (*T. spiralis*), is a globally distributed, re-emerging zoonosis [1]. In 55 countries worldwide, human

trichinellosis has been reported and is a major socio-economic burden in some developing countries [2, 3]. In Egypt, human trichinellosis is present; the prevalence of *T. spiralis* larvae infection in

slaughtered pigs was 1.08 % in Albasatin slaughterhouse, Cairo Governorate (Egypt) [4]. Severity of the clinical picture depends on the species of the parasite, the number of living larvae ingested, the host's age, sex and immune status. There are intestinal, parenteral and muscular phases of trichinellosis, characterized by fever, malaise, anorexia, nausea, vomiting, abdominal pain and diarrhea, which may be so severe that increased mortality due to dehydration [5]. The striated muscles are mainly the muscles affected during the parenteral phase. Dyspnea, respiratory failure, myocarditis, cardiac failure and diffuse encephalopathy can be complicated [6]. In being the only species that occupies skeletal muscle fibers and modifies them into nurse cells, *T. spiralis* has a unique nature among all known nematodes. The parasite needs to recruit new blood vessels to the developing nurse cell in order to maintain a long-term host-parasite relationship and maintain its metabolic activity to meet the ever-growing demand for nutrients [7]. This is accomplished by initiation of angiogenesis this [8]. In all vascularized organs, pericytes are perivascular cells found in abundance. They control numerous functions, including the growth of vessels and angiogenesis [9]. Raza *et al.* (2010) [10] reported that angiogenesis modulation through the promotion of endothelial cell survival and migration is the main function of the pericyte in both skeletal and cardiac muscle. Expression of CD34 and CD31 has been used to identify and isolate pericytes from skeletal muscle [11, 12]. These markers have also been shown, more specifically, to induce therapeutic angiogenesis in animal models. In addition, *T. spiralis* infection induces the production of Vascular Endothelial Growth Factor (VEGF), which is a potent angiogenic stimulator. Both VEGF mRNA and VEGF peptides were detected in the developing nurse cell cytoplasm [13]. Moccia *et al.* [14] showed that the flux of calcium ions has many biological functions

that promote angiogenesis and modulate it. Its effect on angiogenesis may change the environment needed for the formation of *Trichinella* nurse cells. Furthermore, in the synthesis and release of inflammatory chemical mediators, calcium ions play a significant role [15, 16]. Several anti-parasitic drugs are used to eliminate adults in the intestinal stage. Benzimidazoles, the most famous of which is mebendazole, are the most common anthelmintics. For children less than 2 years old, benzimidazole derivatives are not recommended and are poorly absorbed from the gastrointestinal tract [17]. Moreover, bioavailability, high degree of resistance and weak activity against encapsulated larvae are limiting factors [18]. Nifedipine is a blocker of the calcium channel that induces vaso-relaxation by blocking the influx of calcium ions into cells of vascular smooth muscle. It is used to decrease systemic blood pressure and to prevent coronary vasospasm by enhancing the functions and number of circulating endothelial progenitor cells that affect new vessel formation; nifedipine improves vascular endothelial function in hypertensive patients [19, 20]. In the present study, the impact of nifedipine as one of CCB on muscular phase (muscle of diaphragm) of murine trichinellosis was assessed by parasitological and histopathological parameters.

2. Materials and Methods

2.1 Experimental animals:

The present study was conducted on 45 males, laboratory bred Swiss albino mice, pathogen-free, 20-25 g weight and 4-6 weeks age, obtained from Theodor Bilharz Research Institute (TBRI), Cairo. The mice were kept on a standard diet under a temperature of 24°C. The study was approved by the ethical committee of the Faculty of Medicine for Girls, Al-Azhar University and by the ethical community of animal research at TBRI and was

conducted in accordance with the international guidelines.

2.2 Experimental design:

The mice were divided into three main groups. **Group (I):** non-infected control group containing 15 mice. **Group (II):** infected non-treated group containing 15 mice. **Group (III):** infected group treated with nifedipine containing 15 mice.

2.3 Experimental infection:

Each mouse was infected orally with about 250 ± 50 *T. spiralis* infective larvae, Egyptian strain (TSL) obtained from (TBRI) [21, 22, 23].

2.4 Drug administration:

Nifedipine (Epilat, 20mg tablets, EPICO, Egypt) was dissolved in distilled water and the doses were calculated by extrapolation of human therapeutic doses to animal doses [24]. Nifedipine was administered on day one (PI) till day 35 (PI) at a dose of 80 μg /mouse /day orally [25].

2.5 Animal sacrifice:

Sacrification of mice was done, according to Denham, (1965) and Gamble, (1996) [21, 22]. on day 35 (PI) after completing the treatment, to determine the impact of the used drug on larvae in the muscular phase.

2.6 The following parameters were evaluated

2.6.1 Parasitological examination:

Detection and counting of muscle larvae: The diaphragm of infected non- treated and infected treated mice was carefully dissected in each mouse, examined under the microscope and the number of encysted larvae was counted per gram of muscle tissue according to Denham, (1965) and Gamble, [21, 22].

2.6.2 Histopathological examination:

Skeletal muscle specimens from the diaphragm were taken from mice sacrificed on 35th day (PI) [26, 27]. These specimens were fixed in 10% formalin, dehydrated and then embedded in paraffin blocks. Paraffin sections were cut into 5 μm thickness, stained by haematoxylin and eosin [28] and examined microscopically to detect histopathological changes.

2.7 Statistical analysis of collected data:

Statistical analysis was done using SPSS program (statistical package of social science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Median and range were calculated to measure central tendency and dispersion of quantitative data. Comparing groups was done using Kruskal-Wallis test to determine the significance in the difference between two medians.

3. Results

3.1 Parasitological results:

Thirty five days after treatment with nifedipine, number of *T. spiralis* larvae/g of muscle was 370 larvae/g indicating a 67.96 % reduction in comparison to the larval count in the infected non-treated group (GI), which was 1150 larvae/g. The reduction was of statistical significance at $P < 0.01$ (Table 1).

3.2 Histopathological results:

Histopathological examination of sections of diaphragm of the non- infected control group showed normal spindle shape muscle fiber with blue nuclei and pink matrix (Figure 1) while sections of diaphragm of the infected non-treated group showed encysted larvae surrounded with cellular infiltrate (Figure 2). In nifedipine-treated group, histopathological examination showed a mild reduction of

inflammatory cellular infiltrate with intact larvae and capsule (Figure 3).

Table (1): Median and range of the number of *T. spiralis* larvae/g of muscle of mice in infected non-treated group versus nifedipine-treated group.

Varieties Animal groups	Number of <i>T. spiralis</i> larvae/g of muscle of different groups		Percentage of reduction	P value
	Kruskal-Wallis test			
	Median	Range		
Group II (infected non-treated group)	1150	800 – 1300	67.96 %	0.004*
Group III (infected treated with nifedipine)	370	270 – 420		

* Highly significant difference (P value <0.01).

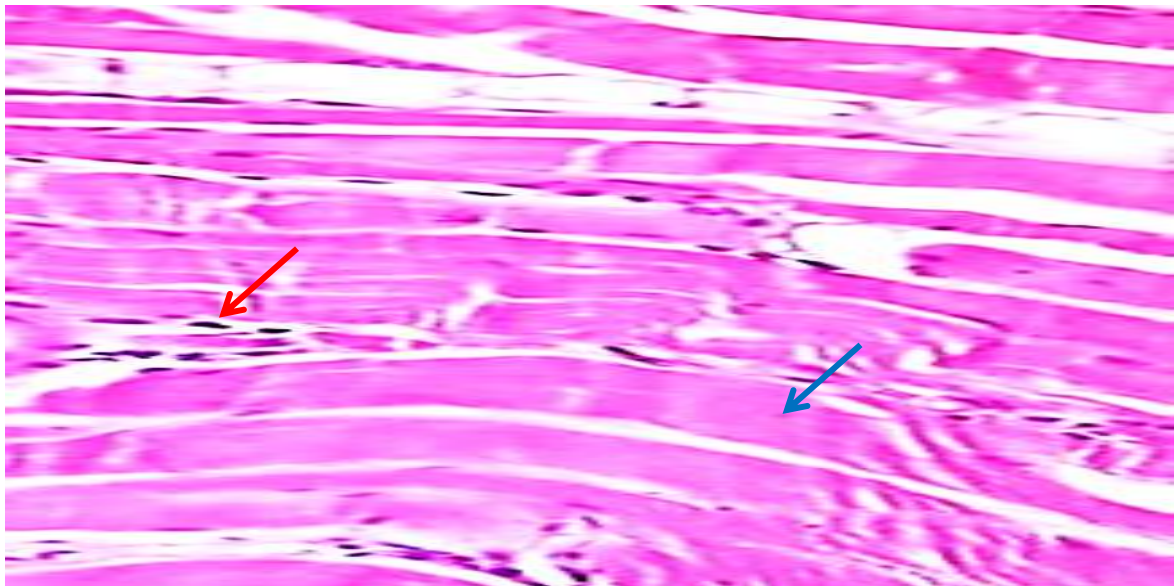


Figure (1): Non-infected control group (GI) showed normal spindle shape muscle fiber with blue nuclei (red arrow) and pink matrix (blue arrow) (H&E 200x).

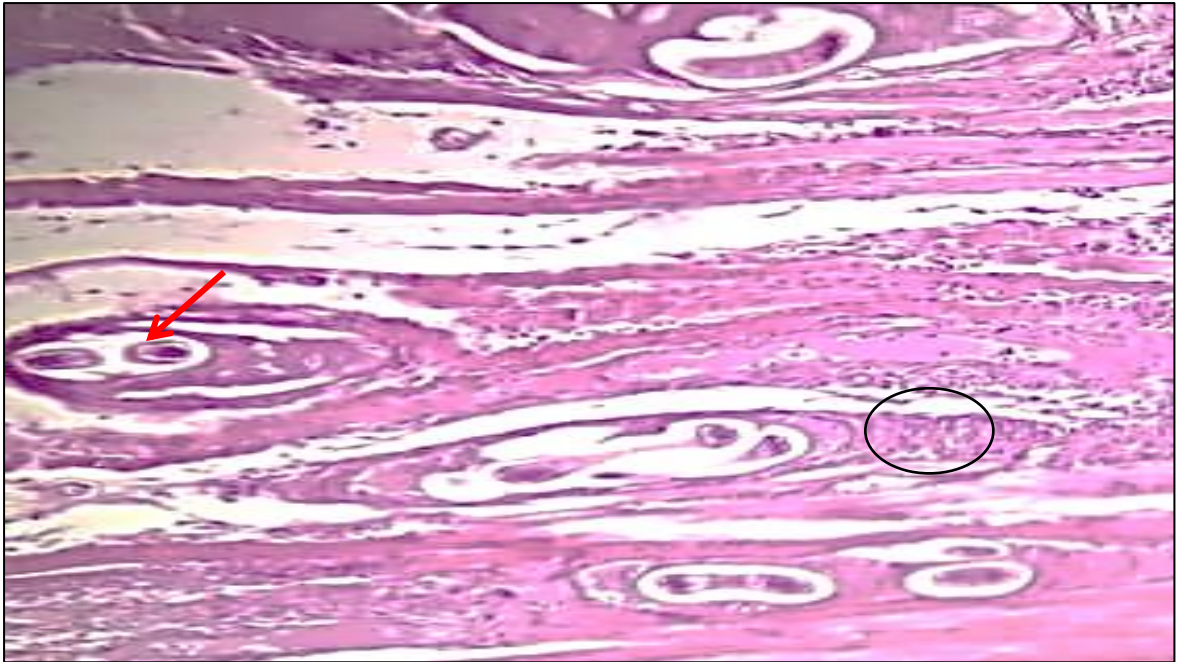


Figure (2): Infected non-treated group (GII) showing inflammatory cellular infiltration surrounding the encysted larvae including lymphocytes, eosinophils, macrophages and some neutrophils (black circle). Larvae were coated by a thick complete collagenous capsule with intact larval morphology (red arrow) (H&E 200x).

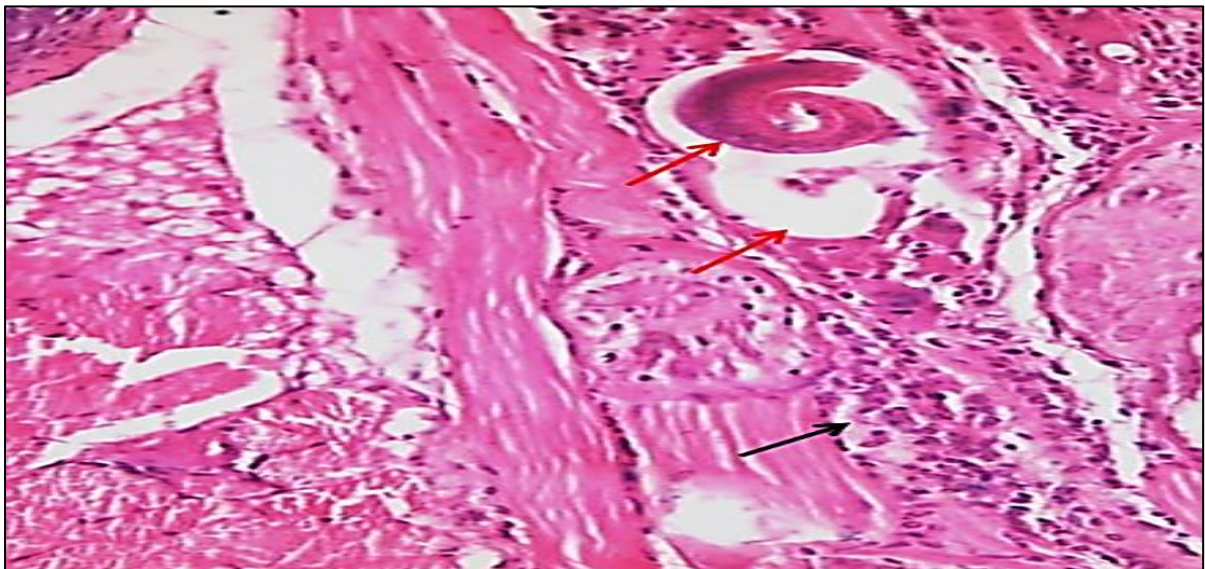


Figure (3): Infected nifedipine-treated group (GIII) showing a mild reduction in the intensity of inflammatory cellular infiltrate (black arrow) with apparently intact larvae and capsule (red arrow) (H&E 200x).

4. Discussion

Trichinellosis is a widespread, serious parasitic disease with significant implications for the medical, veterinary and economic sectors, particularly in developing countries [29].

In addition to occupying and developing inside the muscle cells, *T. spiralis* larvae transform it to be fully at their service after becoming nurse cells surrounded by a vascular network. Subsequently, not only does this network nourishes the parasite, but also protects it against the host immune response [8]. It has been reported that *T. spiralis* induces angiogenesis at the site of tissue infection to supply the required nutrients to the parasite and to act as a reliable waste disposal system by improving the expression during the formation of nurse cells of the chief angiogenic molecule, namely VEGF [8].

Ivermectin (IVM) is an alternative to benzimidazole and has anthelmintic options for the treatment of trichinellosis. It is a powerful macrocyclic lactone that, through increasing the influx of chloride ions across the cell membrane, causes paralysis in nematodes and arthropods [30]. In patients with encysted *Trichinella larvae*, the biochemical environment is affected by other drugs that may be used for the management of co-morbid conditions. Calcium channel blockers (CCB) that are widely used in clinical practice are among these medicines [31]. This research, to the best of our knowledge, is the first to study the effect of a CCB, nifedipine, on the muscular phase of experimental trichinellosis.

In skeletal muscles, calcium channels have an essential role and are important for their contraction. Calcium channel blockers have a direct relaxant effect on the skeletal muscle and decrease the strength of striated muscle contraction by inhibiting the release of calcium from the sarcoplasmic reticulum. In addition, calcium plays an important role in the contraction of blood vessels and angiogenesis [32]. Consequently, CCB may affect the

necessary environmental conditions for the encystation of *Trichinella* larvae. Nifedipine was chosen in the current study to explore the effect of calcium channel blockade on the muscular phase of *T. spiralis* infection. The effects of the drug on infection were evaluated and parasitological and histopathological effects were compared in various studied groups.

As regard to the parasitological assessment, nifedipine at a dose of 80 µg/mouse/day resulted in a substantial decrease in the number of *T. spiralis* larvae in the diaphragm muscle compared to the control group, which was highly significant reduction (67.96 %).

These findings were in accordance with an in vitro study done by Christ and Stillson [33] who revealed that nematode muscle contraction appeared to depend on extracellular calcium of muscle membrane, and that nifedipine reduced the calcium influx across the muscle membrane of *Acanthocheilonema viteae* leading to inhibition of spontaneous isotonic contractions of the filariid parasite leading to flaccid paralysis.

Similar results were also observed in the study conducted by Silva-Moraes *et al.* [34] when nifedipine was added to adult *Schistosoma mansoni* cultures, an impaired or absent motility was observed in male and female worms together with several tegumental injuries. The authors attributed these observations to the blockage by nifedipine of calcium channels located on the external membrane of the *Schistosoma mansoni*, leading to flaccid paralysis of the parasite. Moreover, these findings can be attributed to the nifedipine-induced liberation of nitric oxide by host endothelial cells which plays a crucial role in anti-parasitic immunity and parasite damage [35].

In the current study, encysted larvae surrounded by inflammatory cellular infiltration including lymphocytes, eosinophils, macrophages and some neutrophils were revealed in the

histopathological study. A thick full collagenous capsule with intact larval morphology in the infected group was revealed. These findings were similar to the findings recorded by Attia *et al.* (2015) [29] that showed the presence of a massive number of *T. spiralis* larvae diffusely present in muscles with massive number of chronic inflammatory cells surrounding the larvae.

For nifedipine-treated mice in the present study, histopathological examination of infected and treated mice showed mild improvement in the inflammatory reaction in muscle sections. The anti-inflammatory effects of CCBs were recorded by Chen *et al.* (1999) [36] and Kumar *et al.* (2016) [37] in an experimentally induced pleurisy and granuloma model in rats, where nifedipine was found to have anti-inflammatory effects.

The anti-inflammatory effects of CCBs have been attributed by Khaksari *et al.* [38]

to the inhibition of Ca²⁺ influx that plays a major role in the synthesis and release of inflammatory mediators. The reduction of Ca²⁺ inhibits the activity of phospholipase A2 and phospholipase C, the enzymes responsible for leukotriene synthesis, and stabilizes the integrity of the cell membrane, thereby preventing injury and inflammation of the tissue.

5. Conclusion

Nifedipine; that widely used in cardiovascular diseases, can be used in the treatment of trichinellosis and has shown a high rate of reduction in muscle *Trichinella* larvae and improves pathology so that it can be used as an adjuvant therapy to treat trichinellosis. In order to achieve alternative therapeutic options for the management of this parasitic infection, further studies on their various types should be carried out.

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